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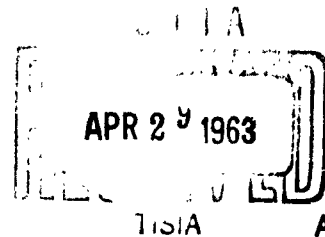
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DIFFERENTIAL RESPONSE TO ALLOGENIC AND
XENOGENIC SKIN GRAFTS BY SUBLETHALLY IRRADIATED (670 rad) AND
NON-IRRADIATED MICE SENSITIZED BY VARIOUS MEANS

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ABSTRACT

Twelve to 14 week old female LAF₁ mice were pre-sensitized either with 3 i.p. injections of BALB/c or rat spleen or skin cells, or by means of two consecutive BALB/c or rat skin tail grafts. One week following the last injection or the rejection of the second skin graft, the mice either were grafted with LAF₁[□], BALB/c, C3D/2 and rat skin or they received 670 rad whole body X radiation and were grafted immediately thereafter. The data indicate that skin grafts induce a more vigorous and more radioresistant "second-set" response than do dissociated cells. Pre-sensitization with allogenic spleen cells resulted in prolonged survival of subsequent allogenic skin grafts in sublethally irradiated mice. The second-set response to a xenogenic skin graft was found to be more radioresistant than was that to an allogenic graft. The converse was true with regard to the first-set response.

SUMMARY

The Problem:

It is generally accepted that any tissue possesses antigens capable of sensitizing a non-related animal to subsequent skin grafts from the original tissue donor. However, it is also apparent that sensitization with a skin graft produces a more vigorous and persistent sensitivity than does immunization by any other means. Presented are data contrasting, in non-irradiated and sublethally irradiated mice, the degree of sensitization to subsequent skin grafts induced by the intraperitoneal injection of spleen cells or dissociated-skin cells, allogenic (another strain of mouse) or xenogenic (another species), with that produced by skin grafts.

The Findings:

Twelve to 14 week old female LAF_1 mice were pre-sensitized either with 3 i.p. injections of BALB/c or rat spleen or skin cells, or by means of two consecutive BALB/c or rat skin tail grafts. One week following the last injection or the rejection of the second skin graft, the mice either were grafted with LAF_1 , BALB/c, C3D/2 and rat skin or they received 670 rad whole body X radiation and were grafted immediately thereafter. The data indicate that skin grafts induce a more vigorous and more radioresistant "second-set" response than do dissociated cells. Pre-sensitization with allogenic spleen cells resulted in prolonged survival of subsequent allogenic skin grafts in

sublethally irradiated mice. The second-set response to a xenogenic skin graft was found to be more radioresistant than was that to an allogenic graft. The converse was true with regard to the first-set response.

INTRODUCTION

It is generally accepted that any nucleated cell, regardless of tissue origin, possesses transplantation isoantigens capable of eliciting specific "homograft sensitivity" (1,2). However, if "homograft sensitivity" is induced by means other than the transplantation of living tissue grafts, the route of administration, amount of isoantigen and schedule of immunization assume prime importance with respect to the results achieved (3-6). In general, sensitization by means of a foreign skin graft produces a more vigorous and persistent "homograft sensitivity" than does sensitization by means of dissociated cells of any type, administered by any route.

In specific instances, "hyperimmunization" with dissociated lymphoid cells or by the intravenous administration of dissociated epidermal cells has resulted in prolonged survival of subsequent specifically related skin grafts (4,6). For more specific detail with regard to this complex subject the reader is referred to the excellent papers cited and to reviews by Lawrence (8), Snell (9) and Brent (10).

Presented below are data contrasting the responses to subsequent allogenic and xenogenic skin grafts by sublethally X irradiated and non-irradiated mice previously sensitized with allogenic or xenogenic skin grafts or with dissociated cell preparations. It will be demonstrated that the degree of sensitization produced by means of dissociated cells (with the methods used) is both less pronounced and

more "radiosensitive" than is that produced with skin grafts. Further, the "homograft sensitivity" induced by xenogenic dissociated skin and spleen cells will be shown to be more vigorous and less "radiosensitive" than that produced with similar preparations of allogenic tissues. It will be apparent the second-set response to a xenogenic skin graft is more radioresistant than is the second-set response to an allogenic graft (11). The converse will be shown to be true with regard to the first-set response.

MATERIALS AND METHODS

Twelve to 14 week old female (C57L x A) F_1 , (LAF $_1$) mice were used as skin graft recipients. Skin graft donors were adult female LAF $_1$ (H2 ab), male BALB/c (H2 d) and (C3H x DBA/2) F_1 , (C3D/2), (H2 Kd) mice, and 2-3 week old male and female Sprague-Dawley rats. The orthotopic tail skin grafting method of Bailey and Usoma was used (12). Details of grafting and the criteria of rejection (total destruction of the engrafted tissue) have been reported previously (11). Mean survival time of the grafts and standard deviation (S.D.) are reported.

Dissociated spleen cells were prepared from the spleens of adult male BALB/c mice and adult female Sprague-Dawley rats. The spleens were removed aseptically and lightly homogenized in cold Tyrode's solution. Dissociated skin cells were prepared from skin removed from the ears of male BALB/c mice or from the tails of 2-3 week old male and female Sprague-Dawley rats. The skin was trimmed of all fat or

cartilage, weighed, and gently but thoroughly homogenized in cold Tyrode's solution. Initially each mouse received the equivalent of 1/5 BALB/c spleen (approx. 20 mg) or 1/20 rat spleen (approx. 30 mg) per injection, but when a direct comparison was being made with dissociated skin cells as an antigenic source, wet weight of the splenic tissue or skin was the measure (22 mg or 50 mg). The mice were sensitized to BALB/c or rat skin with two consecutive skin grafts, or to dissociated spleen or skin cells by means of 3 intraperitoneal injections of the respective homogenate within 9 days.

One week following the rejection of the second skin graft or the last injection of dissociated skin or spleen cells, the mice either were grafted with LAF₁, BALB/c, C3D/2 and rat skin, or they received 670 rad whole body X radiation (LD 5) and were grafted within 6 hours thereafter. The radiation factors (250 KVP, 15 ma; HVL 1.5 mm Cu; 30 rad/min) and details of exposure were the same as previously reported from this Laboratory (13). Norms were obtained for first and second-set responses of non-irradiated LAF₁ mice to (1) BALB/c skin grafts alone, (2) rat skin grafts alone and (3) BALB/c, C3D/2 and rat skin grafts. As controls, non-sensitized mice received 670 rad whole body X radiation and were grafted with LAF₁, BALB/c, C3D/2 and rat skin within 6 hours thereafter.

In order to compare the degree of sensitization induced by dissociated skin or spleen cells, allogenic or xenogenic, groups of

mice received, within 9 days, 3 intraperitoneal injections of the respective homogenate (22 mg or 50 mg wet weight of tissue per injection). One week following the last injection, they either were grafted or they received 670 rad whole body X radiation and were grafted as described above.

Certain groups of mice were regrafted 90 days after the original full set of grafts were in place. This was done in an effort to determine if the original method of sensitization (if any) and/or the subsequent irradiation (if any) would influence or modify the degree of sensitization induced by the test grafts.

All mice were housed 10 per cage. The diet was Purina Lab Chow, and water containing 1% Neomycin was given ad lib.

RESULTS

Non-Irradiated Mice Table I.

Non-irradiated LAF₁ mice rejected first-set BALB/c skin grafts in 12.6 ± 0.8 days and second-set grafts in 6.1 ± 1.2 days. First-set rat skin grafts were rejected in 7.6 ± 0.7 days and second-set grafts in 4.4 ± 0.6 days. When a full set of grafts (LAF₁, BALB/c, C3D/2 and rat) were placed on non-sensitized mice there was a slight acceleration of the first-set response to the allogenic grafts (10.1 ± 1.6 days), but no effect was noted on the rejection time of the rat grafts. The second-set response to a full set of grafts was essentially that seen when each graft was tested separately. Mice pre-sensitized with BALB/c

TABLE I
REJECTION OF ALLOGENIC AND XENOGENIC SKIN GRAFTS BY LAF₁ MICE
PREVIOUSLY SENSITIZED WITH SKIN GRAFTS OR DISSOCIATED SPLEEN CELLS¹

ORIGINAL MEANS OF SENSITIZATION	PRIOR GRAFTS OR AMT. OF SPLEEN	NUMBER OF MICE	ISOGRAFTS REJECTED	TIME OF COMPLETE REJECTION MEAN SURVIVAL(days) ± S.D.		
				BALB/c	C3D/2	RAT
None		33		12.6 ± 0.8		
None	1	38		6.1 ± 1.2		
None		30	0	10.1 ± 1.6	10.1 ± 1.5	7.7 ± 1.0
None	1(full set)	10	0	7.1 ± 0.3	7.0 ± 0.7	4.0 ± 0.0
BALB/c skin graft	2	10	0	7.1 ± 1.0	7.2 ± 0.9	7.7 ± 0.6
BALB/c spleen cells	22 mg x 3*	14	0	11.4 ± 2.8	10.7 ± 1.5	8.5 ± 0.7
None		28				7.6 ± 0.7
None	1	29				4.4 ± 0.6
Rat skin graft	2	18	0	9.0 ± 1.0	9.0 ± 1.0	3.6 ± 0.5
Rat spleen cells	22 mg x 3	9	0	10.5 ± 1.8	11.1 ± 1.3	5.0 ± 0.0
Rat spleen cells	1/20 x 3	11	0	9.9 ± 1.4	10.0 ± 0.8	8.4 ± 0.9

¹ Orthotopic tail skin grafts, single or multiple, were placed on 12-14 week old female LAF₁ mice. Tail skin donors were BALB/c and C3D/2 adult male mice, 2-3 week old male and female Sprague-Dawley rats. Unless specified, the mice were pre-sensitized either with two consecutive allogenic or xenogenic skin grafts or 3 i.p. injections (within 9 days) of an homogenate of rat or BALB/c spleen cells. One week following the last injection of spleen cells or the rejection of the second skin graft, the mice were grafted with LAF₁, BALB/c, C3D/2 and rat skin.

*Net weight.

skin grafts rejected subsequent BALB/c and C3D/2 grafts in a normal second-set manner, while rat skin grafts were rejected as first-set grafts. However, mice pre-sensitized with BALB/c dissociated spleen cells (22 mg or 50 mg) rejected the allogenic skin grafts as if they were first-set, i.e., there was no evidence of accelerated rejection (Table I and III).

Mice previously sensitized with rat skin grafts rejected their allogenic skin grafts (first-set) in an accelerated manner (9.0 ± 1.0 days). (A previous report (11) has shown that no common transplantation antigens are shared between rat skin and BALB/c or C3D/2 bone marrow cells.) However, pre-sensitization with rat spleen cells produced not only a less vigorous "second-set" response to rat skin grafts (5.0-5.2 versus 3.6-4.4), but little or no effect upon the rejection of first-set allogenic grafts (Table I and III). Moreover, in one instance (Table I), sensitization with rat spleen cells produced a slight prolongation of survival of first-set rat grafts (8.4 ± 0.9 days).

Sublethally Irradiated (670 rad) Mice Table II.

A significant number of sublethally irradiated mice previously sensitized with BALB/c skin grafts rejected subsequent allogenic grafts appreciably sooner than did the control group. While one group of mice differed only slightly from the controls, 50% of a second group rejected their allogenic grafts between the seventh and ninth post-irradiation days. However, mice pre-sensitized with BALB/c spleen cells had great

TABLE II

REJECTION OF ALLOGENIC AND XENOGENIC SKIN GRAFTS BY SUBLETHALLY IRRADIATED (670 RAD) LAF₁ MICE
PREVIOUSLY SENSITIZED WITH SKIN GRAFTS OR DISSOCIATED SPLEEN CELLS*

MEANS OF SENSITIZATION	PRIOR GRAFTS OR AMT. OF SPLEEN	NUMBER OF MICE	ISOGRAFTS REJECTED	TIME OF COMPLETE REJECTION MEAN SURVIVAL(days) ± S.D.		
				BALB/c	C3D/2	RAT
None		21	2	21.0 ± 3.5	21.1 ± 3.3	22.7 ± 3.5
BALB/c skin grafts	2	6	0	18.3 ± 3.1**	18.2 ± 4.4	22.1 ± 2.2
BALB/c skin grafts	2	10	1	10.7 ± 3.4	10.8 ± 3.1*	24.4 ± 2.3
BALB/c spleen cells	1/5 x 3	19	0	28.0 ± 5.7	28.8 ± 5.1	21.8 ± 2.8
Rat skin grafts	2	7	1	22.1 ± 3.0	22.2 ± 3.0	7.0 ± 1.4
Rat skin grafts	2	10	1	15.4 ± 4.3	11.4 ± 4.9	4.4 ± 0.8
Rat skin grafts	2	9	0	20.5 ± 8.7	18.3 ± 8.0	3.6 ± 0.6
Rat spleen cells	1/20 x 3	9	1	27.6 ± 8.0	27.4 ± 7.0	10.5 ± 4.8
Rat spleen cells	1/20 x 3	10	1	21.3 ± 5.2	21.8 ± 8.4	12.2 ± 4.8

* One week following the last injection of spleen cells or the rejection of the second skin graft, the mice received 670 rad whole body X radiation and were grafted with LAF₁, BALB/c, C3D/2 and Rat skin within 6 hours, thereafter.

** 5/10 BALB/c grafts rejected between 7-9 days.

*5/9 C3D/2 grafts rejected between 7-9 days.

difficulty rejecting their allogenic grafts (Tables II and III). All three groups, so treated, showed significant prolongation of allogenic graft survival as compared to the controls. In only one instance, in a group of 4 mice pre-sensitized with BALB/c spleen cells, was the rejection of the rat grafts delayed (Table III).

Two of three groups of mice, previously sensitized with rat skin grafts and sublethally irradiated, rejected subsequent rat grafts in a normal second-set manner. In the third group, rejection of the rat grafts was slightly delayed (7.0 ± 1.4 days). One group of mice, so treated, rejected concurrent allogenic grafts significantly sooner than did the control group. While mice sensitized with rat spleen cells did reject subsequent rat grafts decidedly sooner than did the controls, the response, which seemed to be dependent upon the amount of antigen given, was neither as vigorous nor as rapid as that seen in mice sensitized with skin grafts (Tables II and III). Moreover, there was little or no effect upon allogenic graft survival (if anything, a slight prolongation of survival).

It should be noted that the first-set response to allogenic skin grafts recovered from the effects of sublethal irradiation significantly sooner than did the first-set response to a xenogenic skin graft.

Dissociated Skin and Spleen Cells Table III.

The rejection of allogenic and xenogenic skin grafts by sublethally irradiated and non-irradiated LAF₁ mice previously sensitized with dissociated spleen cells, BALB/c or rat, has been described above.

TABLE III
REJECTION OF ALLOGENIC AND XENOGENIC SKIN GRAFTS BY
NON-IRRADIATED AND SUBLETHALLY IRRADIATED (670 RAD) LAF₁ MICE
PREVIOUSLY SENSITIZED WITH DISSOCIATED SKIN OR SPLEEN CELLS*

MEANS OF SENSITIZATION	WET WT. MG.	RADS	NO. OF MICE	ISOGRAFTS REJECTED	TIME OF COMPLETE REJECTION MEAN SURVIVAL(days) ± S.D.	
					BALB/c	C3D/2
None			30	0	10.1 ± 1.6	10.1 ± 1.5
None		670	21	2	21.0 ± 3.5	21.1 ± 3.3
BALB/c spleen cells	22 mg x 3		14	0	11.4 ± 2.8	10.7 ± 1.5
BALB/c spleen cells	22 mg x 3	670	9	0	28.3 ± 4.0	26.0 ± 4.6
BALB/c spleen cells	50 mg x 3		10	0	10.1 ± 2.1	10.4 ± 2.0
BALB/c spleen cells	50 mg x 3	670	4	0	30.5 ± 1.1	29.6 ± 2.3
BALB/c skin cells	22 mg x 3	670	5	0	23.0 ± 9.4*	17.5 ± 9.8 [#]
BALB/c skin cells	50 mg x 3		10	0	8.6 ± 1.8	7.2 ± 0.7
BALB/c skin cells	50 mg x 3	670	9	0	21.1 ± 4.1	20.3 ± 4.1
Rat spleen cells	22 mg x 3		9	0	10.5 ± 1.8	11.1 ± 1.3
Rat spleen cells	22 mg x 3	670	9	0	22.2 ± 3.6	20.1 ± 3.1
Rat spleen cells	50 mg x 3		10	0	11.4 ± 1.2	10.3 ± 1.5
Rat spleen cells	50 mg x 3	670	9	0	25.0 ± 3.6	24.2 ± 4.5
Rat skin cells	22 mg x 3	670	9	0	18.4 ± 2.5	20.0 ± 2.7
Rat skin cells	50 mg x 3		9	0	10.7 ± 1.5	9.9 ± 2.2
Rat skin cells	50 mg x 3	670	8	0	22.7 ± 4.2	19.3 ± 8.0

* Mice were sensitized with 3 i.p. injections (within 9 days) of a homogenate of BALB/c or Rat, spleen or skin cells. One week following the last injection, the mice received 670 rad whole body X radiation, and within 6 hours they were grafted with LAF₁, BALB/c, C3D/2 and rat skin.

* 2/5 BALB/c grafts rejected at 9 days.

* 1/5 C3D/2 grafts rejected at 9 days.

Non-irradiated mice pre-sensitized with BALB/c dissociated skin cells rejected subsequent allogenic grafts in an apparently normal second-set manner. However, sublethally irradiated mice, so treated, rejected their allogenic grafts at about the same time as did the non-sensitized irradiated controls, i.e., there was no evidence of accelerated rejection or of prolongation of survival. In addition, the survival of rat skin grafts was somewhat prolonged under these conditions.

Non-irradiated mice pre-sensitized with rat skin cells rejected subsequent rat skin grafts with an apparently normal second-set response. However, sublethally irradiated mice pre-sensitized with rat dissociated skin cells (22mg x 3) rejected rat skin grafts at about the same time as did the control group. When the sensitizing dose was increased (50 mg x 3), the irradiated mice rejected the rat grafts in 9.8 ± 4.4 days (about as effective as an equal weight of dissociated spleen cells). In no instance was there an appreciable effect upon allogenic graft survival.

Irradiated and Non-irradiated Mice Regrafted 90 days After Original Full Set of Grafts Table IV.

Irradiated and non-irradiated, "non-pre-sensitized" mice, regrafted 90 days after the original full set of grafts were in place, rejected all grafts somewhat sooner than did non-irradiated mice 20 days after the first set of grafts. Sublethally irradiated mice, pre-sensitized with BALB/c spleen cells and regrafted 90 days after irradiation, rejected

TABLE IV

REJECTION OF ALLOGENIC AND XENOGENIC SKIN GRAFTS BY PRE-SENSITIZED AND NON-PRE-SENSITIZED, *
SUBLETHALLY IRRADIATED (670 RAD) AND NON-IRRADIATED, LAF₁ MICE 90 DAYS AFTER ORIGINAL GRAFTING

ORIGINAL MEANS OF SENSITIZATION	DOSE (rads)	TIME AFTER IRRAD. OR AFTER 1ST SET (days)	NO. OF MICE	ISOGRAPHS REJECTED	TIME OF COMPLETE REJECTION MEAN SURVIVAL(days) ± S.D.		
					BALB/c	C3D/2	RAT
None			17	2	9.9 ± 1.6	10.2 ± 1.7	7.1 ± 0.6
None**		20	10	0	7.1 ± 0.3	7.0 ± 0.7	4.0 ± 0.0
None		90	7	0	5.9 ± 2.0	4.3 ± 0.9	3.8 ± 0.5
None	670	0.2	21	2	21.0 ± 3.5	21.1 ± 3.3	22.7 ± 3.5
None	670	90	4	0	5.0 ± 1.1	5.0 ± 1.1	4.0 ± 0.0
BALB/c spleen cells			10	1	10.5 ± 2.1	10.5 ± 1.4	8.7 ± 0.6
BALB/c spleen cells*		90	10	2	6.5 ± 0.7	6.2 ± 1.2	3.9 ± 0.3
BALB/c spleen cells	670	0.2	10	0	32.5 ± 3.1	32.2 ± 2.5	22.0 ± 3.0
BALB/c spleen cells	670	90	10	0	4.7 ± 1.7	5.6 ± 2.1	3.6 ± 0.6
BALB/c skin grafts			10	0	7.1 ± 1.0	7.2 ± 0.9	7.7 ± 0.6
BALB/c skin grafts		90	10	0	5.3 ± 1.1	4.9 ± 1.0	3.7 ± 0.6
BALB/c skin grafts	670	0.2	10	1	10.7 ± 3.4	10.8 ± 3.1	24.4 ± 2.3
BALB/c skin grafts	670	90	6	0	6.3 ± 3.0	4.0 ± 0.0	3.8 ± 0.4
Rat spleen cells			9	0	10.5 ± 1.8	11.1 ± 1.3	5.0 ± 0.0
Rat spleen cells		90	9	0	7.0 ± 1.1	6.4 ± 1.4	4.4 ± 1.1
Rat spleen cells#	670	0.2	10	1	21.3 ± 5.2	21.8 ± 8.4	12.1 ± 4.8
Rat spleen cells#	670	90	9	0	5.9 ± 1.1	5.5 ± 1.3	3.7 ± 0.5
Rat skin grafts			10	0	8.8 ± 1.0	9.1 ± 1.0	3.8 ± 0.3
Rat skin grafts		90	9	0	5.7 ± 1.1	5.2 ± 0.7	3.7 ± 0.5
Rat skin grafts	670	0.2	10	1	15.4 ± 4.3	11.4 ± 4.9	4.4 ± 0.8
Rat skin grafts	670	90	4	0	7.2 ± 1.2	7.0 ± 2.4	4.0 ± 0.0

* The mice were regrafted 90 days after the original full set of grafts were in place. Previous means of sensitization (if any) and amount of X radiation (if any) are outlined in the table. The results obtained upon regrafting are paired with the initial results from the same mice.

** Second-set to all grafts.

* Two isografts were rejected at 6 days. All others appeared to be in stage of early rejection between 4th and 6th days.

All isografts were very inflamed and friable between the 4th and 6th days.

all grafts somewhat sooner than did a similar group which had not been irradiated (4.7 ± 1.7 days versus 6.5 ± 0.7 days). These results held true, generally, for all groups, irradiated and non-irradiated, which were regrafted 90 days after the original full set of grafts. One can conclude, therefore, that neither prior methods of sensitization nor sublethal irradiation interferes materially with the subsequent sensitization induced by means of skin grafts.

During the course of the experiments previously mentioned, it became apparent that, during a vigorous second-set response to allogenic or particularly to xenogenic skin grafts, the concurrent isografts and allografts become markedly reddened and friable. This effect was most prominent during the last series of experiments (regrafting 90 days after the original full set of grafts were in place). In one group of 10 mice (Table IV) two isografts were rejected at 6 days in a manner suggestive of a "homograft response", and the other isografts seemed to be in imminent danger of succumbing to a similar fate. In a second group of nine mice, a similar phenomenon was observed, although no isografts were actually lost; the reddening and friability quickly subsided after the sixth day, i.e., after rejection of the allogenic and xenogenic skin grafts. The reaction appeared to be predominantly vascular in nature, being characterized by petechial hemorrhages, capillary fragility and, at times, frank ecchymoses. The nature of the pathological changes, as observed at the graft sites, strongly suggested either

the presence of a non-specific agent capable of greatly increasing capillary permeability or a deficiency of a factor essential to the maintenance of capillary wall integrity. All surviving isografts were intact and healthy 60 days after being placed. This phenomenon was not observed among the control groups.

DISCUSSION

The foregoing data, derived from non-irradiated and sublethally irradiated mice, indicate that with the methods used dissociated skin or spleen cells induce a "homograft sensitivity" inferior to or perhaps unlike that produced with skin grafts, allogenic or xenogenic. Non-irradiated mice pre-sensitized with allogenic dissociated skin cells or with xenogenic dissociated skin or spleen cells, rejected subsequent skin grafts of the appropriate genotype in an apparently normal second-set manner. By contrast, sublethally irradiated mice, similarly treated, rejected their grafts significantly later than did mice pre-sensitized with skin grafts. Moreover, sublethally irradiated mice pre-sensitized with allogenic spleen cells rejected subsequent allogenic grafts significantly later than did the non-sensitized sublethally irradiated controls. The latter phenomenon suggests "self enhancement" mediated through humoral antibodies (5). In general, sensitization with dissociated rat cells resulted in a more vigorous response to subsequent skin grafts by non-irradiated and sublethally irradiated mice than did sensitization with comparable allogenic tissues.

While it is not possible from these data to clearly distinguish quantitative from qualitative differences in the "homograft sensitivity" induced by the several methods used, the disparity between the degree of sensitivity elicited by dissociated cells and orthotopic skin grafts can hardly be a matter of antigenic dosage, as the total amount of tissue administered with the former method was up to forty times greater than with the latter. One therefore is inclined to believe the "immune system" deals with dissociated cell antigens in a manner qualitatively different from that reserved for solid homografts, i.e., a true "second-set" response is not, as a rule, induced by means of dissociated cells.

A previous report (11) presented data demonstrating, in lethally irradiated, bone-marrow protected mice, the differential radiosensitivity of first and second-set responses to allogenic and xenogenic skin grafts: that is, the second-set response of mice pre-sensitized with allogenic or xenogenic skin grafts was more radioresistant than was the first-set response; the second-set response to a xenogenic skin graft was more radioresistant than was that to an allogenic graft; the converse appeared to be true with regard to the first-set response. The present data in sublethally irradiated mice support and extend these findings. The implications of these observations with regard to the possible heterogeneity of the "immune system" have been discussed previously (11): These data strongly suggest the existence of independent and interdependent "cell lines or systems", each with its own spectrum and potential for reactivity.

The data contained in the present report suggest, further, that "sensitivity" induced by means other than skin grafts is the manifestation of a mechanism qualitatively different from the classic second-set response.

SUMMARY

1. Data are presented demonstrating in sublethally X-irradiated (670 rad) mice the differential radiosensitivity of the first and second-set responses to allogenic (H-2 difference) and xenogenic (rat) skin grafts: The second-set response is more radioresistant than is the first-set response; the second-set response to a xenogenic skin graft is more radioresistant than is that to an allogenic graft; the converse is true with regard to the first-set response.

2. The "homograft sensitivity" induced with xenogenic or allogenic dissociated skin or spleen cells (with the methods used) appears to be "inferior" to or unlike that induced with skin grafts. Sensitization with xenogenic dissociated cells resulted in a more vigorous response (i.e., shorter rejection time) to subsequent appropriate grafts in both non-irradiated and sublethally irradiated mice than did sensitization with comparable allogenic tissues.

3. Allogenic skin grafts survived significantly longer on sublethally irradiated mice, pre-sensitized with allogenic dissociated spleen cells than they did on the appropriate controls. By contrast, pre-sensitization with allogenic dissociated skin cells had essentially no effect on allogenic graft survival in sublethally irradiated mice.

4. Non-irradiated and sublethally irradiated (670 rad) mice previously sensitized with rat skin grafts rejected subsequent first-set allogenic grafts significantly sooner than did their appropriate controls. This effect was not seen when the mice had been previously sensitized with dissociated rat skin or spleen cells.

5. Neither the initial method of sensitization nor sublethal irradiation influenced the sensitization induced by subsequent skin grafts.

6. Certain observations made during the accelerated rejection of allogenic and particularly of xenogenic skin grafts suggested either the presence of a non-specific agent capable of disrupting the capillary bed at the graft sites or the deficiency of a factor essential to the maintenance of capillary wall integrity.

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